APPLICATION

FOR

UNITED STATES LETTERS PATENT

BY

CHARLES T. ESMON

AND

JUN XU

FOR

TARGETING OF MOLECULES TO LARGE VESSEL ENDOTHELIUM USING EPCR

TARGETING OF MOLECULES TO LARGE VESSEL ENDOTHELIUM USING EPCR

The United States government has certain rights in this invention by virtue of National Heart, Lung and Blood Institute of the Institutes of Health grant No. P01 HL54804 to Charles T. Esmon.

Background of the Invention

The present invention is generally in the area of targeting delivery of nucleotides and other molecules to large vessel endothelium using the endothelial cell protein C/activated protein C receptor ("EPCR").

Endothelial cells are a primary defense mechanism against cellular infiltration and thrombosis. Abnormal function of the endothelial cells contributes to myocardial infarction (MI), stroke and the development of atherosclerotic plaque. Atherosclerosis and most other vascular disease primarily occur in large vessels. In particular, large vessels are prone to a variety of diseases leading to atherosclerosis and thrombosis, resulting in heart attacks, strokes, deep vein thrombosis and pulmonary emboli. Gene therapy to modify the vascular lesions would be desirable.

Unfortunately, targeting endothelial cells non-specifically is often inadequate. Since more than 95% of endothelial cells are in the capillaries, arterioles and postcapillary venules, any therapy directed toward endothelial cells *per se* runs the risk of systemic complications. One must be confident that the gene expression is limited to the desired cells when using a gene therapy approach. However, no means for specifically targeting delivery to large vessel endothelium, in contrast to small vessel endothelium, or other types of tissues, are known.

It is therefore an object of the present invention to provide a means for specifically targeting delivery to large vessel endothelium.

It is a further object of the present invention to provide a means for delivering an agent to the endothelial cell nucleus of large vessels.

Summary of the Invention

EPCR is located primarily on the surface of endothelial cells of large vessels. It translocates from the cell surface to the nucleus, and can be used to direct uptake by the cells of molecules that bind to EPCR and materials covalently coupled to the EPCR binding molecules. Molecules which bind to the EPCR, such as activated protein C ("APC"), but not protein C ("PC"), will be transported specifically to the nucleus of endothelial cells, especially those of the large vessels. Molecules which bind to the EPCR, such as PC, will direct molecules to endothelial cells, especially those of the large vessels. Molecules to be delivered can be nucleic acid, such as DNA, proteins such as transcription factors, diagnostic agents or other types of drugs. Conjugates of the materials to be administered can be formed by ionic or covalent coupling, for example, through direct conjugation of an anti-EPCR monoclonal antibody to a protein, including fusion proteins, or through the covalent attachment of a positively charged polymer such as polylysine to an anti-EPCR antibody, where the positively charged polymer binds nucleic acid or other negatively charged molecules by ionic charges. Steptavidin and biotin can also be used to conjugate molecules to the anti-EPCR antibody.

Examples demonstrate selective transport to the nucleus mediated by antibodies to EPCR, transport of proteins and genes bound to the antibodies to EPCR by charged polymers and steptavidin-biotin coupling, and transport selectively of APC, but not PC, to the nucleus of large vessel endothelial cells.

Brief Description of the Drawings

Figure 1 is a graph of reporter gene transfer via a DNA anti-EPCR mAb-poly-L-lysine complex. Luciferase gene expression was measured in EA.hy926 cells transfected with the DNA-mAb conjugate as described in the experimental procedures. Control is the EA.hy926 cells transfected with same amounts of DNA, mAb and poly-L-lysine.

Figure 2 is a graph of ^{125}I streptavidin nuclear delivery via biotinylated anti-EPCR mAb EA.hy926 cells cultured with 13.9 nM 125I labeled streptavidin in the absence (-) or presence (+) of 11.1 nM biotinylated anti-EPCR mAb(JRK1500) for 1 hr at 37° C.

Figure 3 is a graph of EPCR mediated nuclear translocation of APC, but not protein C, in human umbilical vascular endothelial cells ("HUVEC").

Detailed Description of the Invention

I. Delivery System Targeted to Large Vessel Endothelium

A. EPCR

The endothelial cell protein C receptor is ideally suited to target genes or other materials to the endothelial cell population of choice. The protein is expressed at the highest levels on endothelium of large vessels, particularly arteries and is at very low or undetectable levels in capillaries, as described by Laszik, et al., *Circulation* 96, 3633-3640 (1997) and PCT/US97/20364 "Endothelium Specific Expression Regulated by EPCR Control Elements" Oklahoma Medical Research Foundation. Protein C can be seen to be associated with vessels that express EPCR, but not with those that do not.

EPCR was cloned and characterized, as described in PCT/US95/09636 "*Cloning and Regulation of an Endothelial Cell Protein C/Activated Protein C Receptor*" by the Oklahoma Medical Research Foundation and U.S. Patent No. 5,695,993 issued December 9, 1997, to Oklahoma Medical Research Foundation. The protein consists of 238 amino acids, which includes a 15 amino acid signal sequence at the N-terminus, and a 23 amino acid transmembrane region which characterizes the receptor as a type 1 transmembrane protein. The protein binds with high affinity to both protein C and activated protein C (Kd=30 nM), which is a naturally occurring anticoagulant, and binding is calcium dependent. A vector encoding EPCR can be obtained from Invitrogen Corporation, 3985B Sorrento Valley Boulevard, San Diego, California 92121.

The method used herein is preferably used with cells that naturally express EPCR, although it is understood that cells that are genetically engineered to express EPCR can also be targeted using agents which bind EPCR, to obtain uptake by the EPCR-expressing cells of material conjugated to the EPCR-binding molecules.

B. Agents which bind to EPCR

Agents which selectively bind to EPCR can be used to deliver materials selectively to large vessel endothelial cells. Exemplary agents include PC, antibodies and antibody fragments which bind to EPCR, and certain other proteins, including fusion proteins, which include the Gla domain of PC. Selection of the agent can be used to target the molecule to be delivered to either the nucleus or to the cytoplasm of the large vessel endothelial cells. For example, APC directs uptake to the nucleus; PC results in uptake primarily within the cytoplasma and organelles therein.

Antibodies to EPCR, whether they block protein C binding or not, are transported to the nucleus. This is because EPCR translocates to the nucleus, not because of antibody dependent translocation. As described in the examples, polyacrylamide gel electropheresis in detergent ("SDS-PAGE") and autoradiography shows ^{125}I labeled monoclonal antibody ("Mab") deposition in the nucleus as detected in nuclear extracts of the HUVEC. Mab 1500 does not block protein C binding to EPCR whereas Mab 1494 does block this binding. Antibodies are found in the nuclear extract. Control antibodies to Thrombomodulin are not detected in the nuclear extract.

Antibodies which can be used to bind to EPCR include polyclonal and monoclonal antibodies, and fragments thereof, produced by enzymatic cleavage or expression of recombinant nucleic acid molecules encoding the fragments. Methods for making EPCR and antibodies to EPCR are described by Stearns-Kurosawa, et al., J. Biol. Chem. 271, 17499-17503 (1996). Antibodies can be obtained using standard techniques using EPCR, either

isolated from natural or recombinant sources as the immunogen. The antibodies can be used as naturally produced, cleaved enzymatically to yield fragments, or expressed from recombinant genes. Monoclonal antibodies can also be generated using standard techniques.

Recombinant and humanized antibodies can also be made using standard techniques. For example, Pharmacia's (Pharmacia LKB Biotechnology, Sweden) "Recombinant Phage Antibody System" (RPAS), which generates a single-chain Fv fragment (ScFv) that incorporates the complete antigen-binding domain of the antibody can be used to make antibodies. In the RPAS, antibody variable heavy and light chain genes are separately amplified from the hybridoma mRNA and cloned into an expression vector. The heavy and light chain domains are co-expressed on the same polypeptide chain after joining with a short linker DNA which codes for a flexible peptide. This assembly generates a single-chain Fv fragment (ScFv) which incorporates the complete antigen-binding domain of the antibody. Murine ScFv molecules can be "humanized"to further reduce the immunogenic stimulus presented.

Methods for "humanizing" antibodies, or generating less immunogenic fragments of non-human antibodies, are well known. A humanized antibody is one in which only the antigen-recognized sites, or complementarity determining hypervariable regions (CDRs) are of non-human origin, whereas all framework regions (FR) of variable domains are products of human genes. These "humanized" antibodies present a lesser xenograft rejection stimulus when introduced to a human recipient. To accomplish humanization of a selected mouse monoclonal antibody, the CDR grafting method described by Daugherty, et al., *Nucl. Acids Res.*, 19:2471-2476, 1991, can be used. Briefly, the variable region DNA of a selected animal recombinant anti-idiotypic ScFv is sequenced by the method of Clackson, T., et al., *Nature*, 352:624-688, 1991, incorporated herein by reference. Using this sequence, animal CDRs are distinguished from animal framework regions

(FR) based on locations of the CDRs in known sequences of animal variable genes. Kabat, H.A., et al.l, *Sequences of Proteins of Immunological Interest, 4th Ed.* (U.S. Dept. health and Human Services, Bethesda, MD, 1987). Once the animal CDRs and FR are identified, the CDRs are grafted onto human heavy chain variable region framework by the use of synthetic oligonucleotides and polymerase chain reaction (PCR) recombination. Codons for the animal heavy chain CRDs, as well as the available human heavy chain variable region framework, are built in four (each 100 bases long) oligonucleotides. Using PCR, a grafted DNA sequence of 400 bases is formed that encodes for the recombinant animal CDR/human heavy chain FR protection.

Other molecules which can be used include proteins such as activated protein C ("APC"), or other proteins which include the vitamin K specific Gla domain of protein C, to direct binding to EPCR. Activated protein C is targeted to the nucleus and hence can be used to carry other proteins or DNA into the nucleus. In contrast, protein C is internalized by the receptor, but the protein C is not targeted to the nucleus. Nevertheless, protein C could be modified with an expression vector and could serve as a means of delivering genes to large vessel endothelium. The genes could then be targeted to the nucleus by a variety of means including attachment of nuclear targeting amino acid peptide sequences to the DNA such as the SV40 nuclear targeting sequence, described by Sebestyén, et al., *Nature Biotechnology* 16, 80-85 (1998).

C. Molecules to be Delivered

A variety of materials can be delivered, ranging from nucleic acids to proteins to diagnostic agents to drugs. In the preferred embodiment, these materials will be directed to the nucleus, particularly in the case of molecules to be expressed.

Nucleic acid molecules include vectors for expression of a gene or cDNA encoding a particular protein, molecules which inhibit or alter

expression of a particular endogenous nucleic acid molecule, such as an antisense molecule, triplex forming oligonucleotide, ribozyme or external guide sequence for a ribozyme.

Many different genes have been identified as candidates to minimize vascular defects including tissue plasminogen activator, agents to inhibit the expression of leukocyte adhesion molecules, thrombomodulin, nitric oxide synthase, etc. The delivery of proteins or protein expression inhibitors, directly or via gene therapy, specifically to large vessel endothelial cells, is one means for addressing these clinical conditions. For example, the anti-thrombotic potential of endothelium can be increased by delivering agents that prevent thrombosis, such as thrombomodulin, heparin proteoglycans, tissue factor pathway inhibitor (TFPI, a potent inhibitor of the tissue Factor-Factor VIIa-Factor X complex), etc. Fibrinolytic activity can be increased by overexpression of tissue plasminogen activator (tPA) or urokinase. Expression of adhesion molecules such as P-selectin or ICAMs can be inhibited to minimize or decrease leukotye infiltration. Transcription factors can be used to induce expression of particular proteins.

Diagnostic agents include molecules which can be used for diagnostic purposes, including radiolabels, fluorescent labels, enzymatic labels, and other materials.

Drugs include molecules which are not normally transported efficiently, or selectively, into endothelial cells, especially those of the large vessels. These may be antiinflammatories, anticoagulants, anti-cytokines, growth hormones, or of any other purpose for which transport into endothelial cells is desirable.

Immunoprecipitates of surface biotinylated EPCR shown as a function of time demonstrate that, since both the surface labeled EPCR and the antibody EPCR complex translocate to the nucleus, translocation is independent of the antibody and hence any agent that binds tightly to EPCR can be translocated to the nucleus. For instance, a luciferase reporter gene

construct bound to polylysine modified anti-EPCR monoclonal antibodies can be transported to the nucleus as evidenced by the antibody dependent expression of luciferase, as shown by the examples. No expression occurs if the construct is added to antibody that has not been modified with polylysine.

D. Methods of Binding to EPCR

The molecules to be delivered must be coupled to the agents which bind to the EPCR. The molecules can be conjugated directly or indirectly to the agents which bind to the EPCR. Binding can be covalent or ionic. Direct binding can be obtained using standard chemical coupling techniques such as using succinic anhydride and published methodology or by expression of the agent and molecule as a fusion protein. Indirect binding can be obtained through an intermediate molecule, such as a positively charged polymer like lysine (which binds to negatively charged molecules such as DNA), steptavidin which binds to biotin, either of which can be conjugated to the agent binding the EPCR, or expressed as a fusion protein with the agent binding the EPCR, or through the use of a chimeric antibody which binds both the EPCR and the molecules to be delivered.

Examples of useful chimeric antibodies include antibodies which bind both EPCR and DNA and antibodies which bind to both EPCR and whatever other molecule is to be delivered. Chimeric antibodies can be made using standard techniques. For example, chimeric antibodies composed of one variable region of anti-DNA antibody can be fused to one variable region of anti-EPCR antibody. Preferably both antibodies are humanized to minimize the immune response.

An example of protein delivery to the nucleus of large vessel endothelial cells is shown in the examples. Streptavidin was carried into the nucleus on biotinylated anti-EPCR mAB, JRK 1500.

Agents binding to EPCR can be coupled to molecules such as polylysine, polypyrroles, chitosan and other molecules known to ionically

bind to DNA or other negatively charged molecules to be delivered. These are typically polymers including numerous positively charged groups.

II. Methods for Administration

Translocation of cell surface receptors to the nucleus has previously been shown for other receptors, including the receptors for fibroblast growth factor (FGF) (Maher, P.A. *J. Cell Biol.* 134:529-536 (1996)), epidermal growth factor (EGF) (Holt, et al., *Biochem.Pharmacol.* 47:117- 126 (1994; Xie, Y. and M.-C. Hung. *Biochem.Biophys.Res.Comm.* 203:1589-1598 (1994)); insulin (Jiang, L.-W. and M. Schindler. *J.Cell Biol.* 110:559-568 (1990)), interleukin 1 (Curtis, et al. *J.Immunol.* 144:1295-1303 (1990)), growth hormone (Lobie, et al. *J.Biol.Chem.* 269:31735-31746 (1994)). In all of these cases, translocation requires binding of the receptor ligand to the receptor. Many of these ligands have adverse biological effects causing cellular proliferation (FGF and EGF) or inflammation (IL-1) (Curtis, et al. 1990) and hence the agonist is inappropriate for general therapy.

As described herein, a delivery means has been developed which is restricted to large vessel endothelium and hence can serve to deliver genes, transcription factors or other cellular modifiers to the nucleus of the large vessel endothelium specifically where the major complications of many cardiovascular diseases are manifested primarily. In the case of EPCR nuclear translocation, serum stimulates the process, but is not required. This means that enhanced delivery may be obtained during inflammation or coagulation processes, which typically accompany the disorders to be treated, or are more pronounced in the areas of the patient where treatment is desired.

In the preferred embodiment, the conjugate of the agent binding to the EPCR and the molecule to be delivered is administered intravascularly to a patient for administration throughout the blood vessels of the body. In

another embodiment, the conjugate is administered to a region of a body in need of treatment thereof, for example, during angioplasty. Alternatively, the material can be used *in vitro*, to treat isolated cells, which can remain in culture or be returned to a patient in need of treatment thereof. The number of molecules to be administered will be determined empirically, based on the efficiency of uptake, the condition to be treated, the number of cells to be treated, the severity of the condition, and other variables normally considered in determining an effective amount.

The conjugate will typically be administered in an appropriate pharmaceutically acceptable carrier such as phosphate buffered saline, saline, or other materials used for administration of drugs intravenously. Conjugate can be administered alone or in combination with other therapeutic agents, such as anticoagulants, antiinflammatories, vasoconstrictors or other drugs appropriate for treatment of the disease indication.

The conjugate can alternatively be administered in a carrier such as a polymeric gel if applied topically or locally, for example, during angioplasty, particularly if administered using the angioplastic catheter, or directly, during surgery. Such catheters and polymeric materials are known, for example, as described in U.S. Patent Nos. 5,779,673 to Roth, et al., 5,749,968 to Melanson, et al., and 5,698,189 to Rowe, et al. See also WO 96/20732 by Chiron Viagene which discloses polymeric materials such as polybrene to enhance transfer of material into cells to be genetically engineered and WO 96/21470 by Genemedicine, Inc. which describes polymers such as the polyoxyethylene oxides (Pluronics™ and Polaxomers™ sold by BASF) to retain genetic material at a site for transduction, and to increase efficiency of transduction. Other carriers can also be used, such as microparticles which provide for controlled release.

The amount to be administered can be determined based on the in vivo half-life of the conjugate, the efficiency of uptake, and the area to be treated. Treatment can be repeated as necessary, based on clinical

judgement, in view of patient response. A pharmaceutically effective amount is that which achieves a clinical response, depending on the disorder to be treated.

The present invention will be further understood by reference to the following non-limiting examples.

Experimental Procedures

Isolation of Nuclei and Nuclear Extracts

Cells in 100 mm culture dishes were rinsed three times with cold Hank's buffered salt solution, (HBSS), and suspended in 1.5 ml of cold cell lysis buffer (10 mM HEPES, pH7.5, 10 mM KCl, 0.1 mM EDTA, 0.1 mM EGTA, 1 mM DTT, 0.5 mM PMSF) by gentle scraping with a rubber policeman. The cells were allowed to swell on ice for 15 min, then 93.8 μl of a 10% solution of NP-40 was added and the tube was vortexed vigorously for 10 sec. Nuclei were pelleted by centrifugation for 30 sec in a microfuge, and washed twice with cell lysis buffer containing 0.5% NP-40. The nuclear pellets were extracted by nuclear extraction buffer (0.1% NP-40, 20 mM HEPES, pH 7.5, 0.4 M NaCl, 1 mM EDTA, 1 mM EGTA, 1 mM DTT, 1 mM PMSF, 1 μg/ml leupeptin, 1 μg/ml aproptinin, 1 mM benzamidine) at 4 ^{0}C for 1 hr. Nuclear extracts were present in the supernatants after centrifugation at 4^{0}C for 15 min.

Translocation of EPCR from the plasma membrane to the nucleus

Cells in 100 mm culture dishes were labeled for 10 min at RT with 0.5 mg/ml sulfo-NHS-LC- Biotin in HBSS with 1 mM Mg^{2+} and Ca^{2+}, rinsed with HBSS1 M Ca^{2+} and Mg^{2+}, and then cultured with fresh medium at 37^{0}C for the indicated time. The cells were rinsed with ice-cold HBSS, lysed and the nuclear extracts were prepared as described above. The cell lysates without nuclei and nuclear extracts were immunoprecipitated with an anti-EPCR mAb (JRK1496, a murine anti- human EPCR antibody which blocks PC/APC binding to EPCR) resin at 4^{0}C for 2 hr, and the immunoprecipitates were washed with 2 M NaCl, 20 mM Tris-HCl, pH 7.5

with 0.1% Lubrol™ PX twice and 0.15 M NaCl, 20 mM Tris-HCl, pH 7.5 with 0.1% Lubrol™ PX twice, eluted by boiling for 3 min in 50% ethylene glycol, 5 mM MES, pH6.0, 0.1% Lubrol™ PX plus 1X Laemmli sample buffer. Samples were subjected to 10% SDS-PAGE. Western blots were performed using streptavidin conjugated horse radish peroxidase (HRP) and developed with ECL system (Amersham).

Immunofluorescence Microscopy

HUVECs were rinsed with HBSS (1 mM Ca^{2+}, Mg^{2+}), fixed for 5 min with 4% paraformaldehyde in HBSS, and permeabilized for 5 min with 0.05% saponin. Permeabilized cells were incubated with 1% BSA for 30min and then incubated with a mAb anti-EPCR (JRK1501, a murine monoclonal antibody to human EPCR which blocks PC/APC binding to EPCR, 10 g/ml) and a rabbit pAb anti-caveolin (10 μg/ml) in HBSS for 30 min, rinsed with HBSS, stained with FITC-conjugated goat anti-rabbit IgG (10 μg/ml) and Cy3 conjugated goat anti-mouse IgG (10 μg/ml) in HBSS for 30 min, rinsed with HBSS. Slides were mounted with ~~Slow Fade~~ SLOW FADE and sealed with nail polish. Dual labeled cells were examined with a laser scanning confocal microscope. (The Cy3 conjugated goat anti mouse IgG stained the nucleus and caucolae providing confirmation of nuclear localization.)

DNA-mAb complex and transfection

1.7 mg mAb anti-EPCR (JRK1500, an antibody that does not block protein C binding) in 1 ml 10 mM sodium phosphate buffer, pH 7.0 was mixed with 0.25 ml 0.1 M sodium periodate in the same buffer, incubated for 15 min at room temperature (RT). The reaction mixture was buffer exchanged with 1 mM sodium acetate buffer, pH 4.0 on a PD-10 column(Pharmacia), 1.5 ml periodate oxidized mAb (1.4 mg) was collected and mixed with 0.5 ml poly-L-lysine (3 mg, mol. wt.= 20,000) in 20 mM sodium carbonate buffer, pH 9.5, and incubated for 2 hr at RT. Sodium borohydride (0.1 ml, 4 mg/ml) was added and the reaction mixture then incubated on ice water for 1 hr. Free poly-L-lysine was removed by four

cycles of concentration and 10 fold dilution with 10 mM glycine, pH 4.2 using a Millipore BIOMAX-100K™ ultrafilter. The overall yield of mAb-poly-L-lysine conjugate was about 70%.

The DNA-mAb complex was made by mixing 0.625 μg pGL3 vector vector which contains the luciferase insert (Promega) in 50 μl 10 mM HEPES, pH 7.5, 150 mM NaCl (HBS) and 0.42 OD_{280} mAb-poly-L-lysine conjugate in 50 μl HBS and incubating for 30 min at RT. EA.hy296 cells were cultured in 12 well plates in 50 μl 10 mM HEPES, pH 7.5, 150 mM NaCl (HBS) with 0.42 OD_{280} mAb-poly-L-lysine conjugate in 50 μl HBS and incubating for 30 min at RT. EA.hy926 cells were cultured in 12 well plates in Dulbecco's minimal essential medium containing 10% supplemented calf serum to 70% confluency. The media was replaced with 0.9 ml fresh media and 100 μl of the DNA-mAb complex was then added.

Example 1: Transport of Iodinated EPCR mAb into the nucleus of endothelial cells.

Human umbilical vein endothelial cells (HUVEC) were cultured with 30 nM ^{125}I labeled Fab of anti-EPCR mAb (JRK 1500) for 1 hr at 37°C. Approximately 10% of the cell associated anti-EPCR mAb was found in the nuclear extract (4,496 of the 43,944 cpm bound).

Example 2: Transport of biotyinylated EPCR mAb into the nucleus over time.

EPCR nuclear translocation was visualized, at 0, 0.5, 1, 2 and 3 hours. EA.hy926 cells were surface biotinylated and then cultured for the indicated time at 37° C. Cell lysates (excluding the nucleus) and nuclear extracts were immunoprecipitated by an anti-EPCR mAb resin. The immunoprecipitates were subjected to SDS-PAGE under reducing conditions and Western Blotting.

The results show that biotin-labelled EPCR was present in both the nuclear extracts as well as the cell lysates, excluding the nucleus. Maximum nuclear uptake occurred within 2 hrs.

Example 3: Transfer of DNA complexed with poly-L-lysine conjugated with anti-EPCR mAb.

Figure 1 is a graph of reporter gene transfer via a DNA anti-EPCR mAb-poly-L-lysine complex. Luciferase gene expression was measured in EA.hy926 cells transfected with the DNA-mAb conjugate as described in the experimental procedures. Control is the EA.hy926 transfected cells transfected with the same amounts of DNA, mAb and Poly-L-lysine but without polylysine conjugation.

The results demonstrate that there is much higher reporter gene transfer in the transfected cells, establishing that DNA can be transported into cell nuclei using a mAb to EPCR.

Example 4: Biotin labeled EPCR mAb can be used for nuclear delivery of streptavidin conjugated moieties.

Figure 2 is a graph of ^{125}I streptavidin nuclear delivery via biotinylated anti-EPCR mAb. EA.hy926 cells cultured with 13.9 nM ^{125}I labeled streptavidin in the absence (-) or presence (+) of 11.1 nM biotinylated anti-EPCR mAb(JRK1500) for 1 hr at 37° C.

The results demonstrate that the streptavidin can be delivered to the nucleus.

Example 5: APC, but not protein C, is transported to the nucleus of endothelial cells by EPCR.

Figure 3 is a graph of EPCR mediated nuclear translocation of APC, but not protein C, in HUVEC. HUVEC were incubated for 10 min at room temperature with or without 200 nM anti-EPCR mAB JRK 1494, an antibody that blocks protein C and binding and activity. The cells were then incubated with 30 nM ^{125}I labeled APC or protein C for 1 hr at 37°C. Cells were washed with HBSS containing 0.5 mM EDTA to remove surface associated APC/protein C before the nuclei were isolated. The amounts of nuclear APC and protein C were calculated based on the specific activity of the labeled proteins.

In the case of APC, approximately 8% of the cell associated counts were in the nucleus. Neglible counts were associated with the nucleus when protein C was used.

Example 6: Stimulation by Serum of EPCR Translocation.

Serum stimulates EPCR nuclear translocation. EA.hy926 cells were surface biotinylated and then cultured in the absence or presence of bovine serum for 1 hr at 37° C. Cell lysate (excluding nucleus) and nuclear extracts were immunoprecipitated by an anti-EPCR mAb resin. The immunoprecipitates were subjected to SDA-PAGE under reducing conditions and Western Blotting.

The results show that a large portion of the biotin-labelled EPCR is transported into the nucleus. This means that clotting in an area could in some cases increase therapeutic gene/agent uptake.

Modifications and variations will be obvious to those skilled in the art and are intended to come within the scope of the appended claims.